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LUNG CARCINOMA AND MALIGNANT MESOTHELIOMA IN PATIENTS EXPOSED TO THOROTRAST: INCIDENCE, HISTOLOGY AND *p53* STATUS

Michael Andersson[1,3,9], Håkan Wallin[2,5,10], Marzieh Jönsson[5], Lise L. Nielsen[2], Jakob Visfeldt[4], Mogens Vyberg[6], William P. Bennett[7], Virna M.G. De Benedetti[7], Lois B. Travis[8] and Hans H. Storm[1]

Divisions of [1] Cancer Epidemiology and [2] Cancer Biology, Danish Cancer Society; Departments of [3] 0ncology and [4] Pathology, National University Hospital/Rigshospitalet, Copenhagen, Denmark; [5] Department of Occupational and Environmental Medicine, University of Lund, Sweden; [6] Institute of Pathology, Aalborg Sygehus, Aalborg, Denmark; [7] Laboratory of Human Carcinogenesis and [8] Radiation Epidemiology Branch, National Cancer Institute, Bethesda, MD, USA.

In a previous registry-based survey of 999 patients injected with α-emitting $^{232}ThO_2$ (Thorotrast), we identified elevated risks for lung carcinoma and malignant mesothelioma. Since injected Thorotrast is retained lifelong mostly in liver, spleen and lymph nodes, the mesothelial surfaces of these organs are constantly irradiated. Thorotrast-administered patients also perpetually exhale ^{220}Rn, a ^{232}Th-daughter. Study of Thorotrast-exposed patients may, therefore, provide data with regard to carcinogenicity of radon exposure, a current public health concern, as well as the pathogenesis of malignant mesothelioma. The incidence and histologic types of lung carcinoma and malignant mesothelioma within the cohort were examined by review of available histopathologic material and medical records. Further, mutations of the *p53* gene were analyzed whenever possible as it has previously been suggested that radon-associated lung carcinomas exhibit specific mutational patterns. The cumulative risk for lung carcinoma reached 11.0% based on 20 confirmed cases. Nine were small cell lung cancer (SCLC), whereas the expected frequency was 18%. The risk for malignant mesothelioma reached 2.5% based on 7 cases. The actuarial risk of malignant mesothelioma for patients given more than 20 ml Thorotrast was 7.8% compared to 1.4% for patients administered smaller amounts. Seven lung carcinomas and 5 malignant mesotheliomas were analyzed for *p53* mutations; only 1 (in a lung adenocarcinoma) was detected. A possible association between Thorotrast and SCLC is suggested. In addition, a possible dose-response gradient exists for Thorotrast and malignant mesothelioma.

Lung carcinoma ranks among the most common malignancies in both men and women. More than 80% of cases are believed to be caused by cigarette smoking. Results of studies of underground miners, however, show that α-particle irradiation may also induce pulmonary neoplasia, and estimates derived from these data suggest that lower levels of radon exposure within indoor dwellings may account for up to 9% of lung carcinomas in the United States (Lubin *et al.*, 1994). Etiological studies relating the level of radon in houses to the risk of lung carcinoma have, however, not consistently demonstrated any significant relationship, perhaps due to the much lower levels of radon at home and to methodological problems related to quantification of lifetime radon exposure in large populations while taking into account cigarette smoking. Efforts have also been made to distinguish lung carcinomas induced by different agents by morphologic appearance or by molecular biological methods. In a combined analysis of lung carcinomas among survivors of the atomic bombings of Japan and among uranium miners, small cell lung carcinomas (SCLC) seemed more often related to ionizing radiation than adenocarcinomas (Land *et al.*, 1993). These observations are consistent with the finding that as many as 50% of lung carcinomas among underground miners are SCLC (National Research Council, 1988). Analysis of the spectrum of mutations in the *p53* tumor suppressor gene in 19 lung carcinomas among underground miners exposed to moderate levels of radon showed no G:C $\rightarrow$ T:A transversions with a non-transcribed, coding strand bias, which are normally seen in tobacco-related tumors (Vähäkangas *et al.*, 1992). Similar findings were noted in a study of lung carcinoma following radiation treatment for Hodgkin's disease (De Benedetti *et al.*, 1994). Among miners with heavy radon exposure, a specific point mutation at codon 249 has been demonstrated in 16 of 52 non-SCLC, and it was suggested that this mutation might be specifically induced by α-particles (Taylor *et al.*, 1994).

Malignant mesothelioma is a rare disease which usually arises from pleura or (less frequently) from the peritoneum. Many cases are believed to have been induced by inhaled or ingested asbestos fibers, while no other known etiologic agents are considered to be important in its genesis. The frequency of *p53* mutations in malignant mesothelioma is low (approximately 20%) in the very few reported series, and the mutational spectrum is not well characterized (Greenblatt *et al.*, 1994).

Thorotrast, a 20% colloidal solution of $^{232}ThO_2$, is a radiologic contrast agent used during the 1930s–1950s, mostly for cerebral arteriography. Since Thorotrast cannot be excreted to any appreciable extent, it is largely retained within the body, predominantly in the reticuloendothelial system, mostly liver, spleen and red bone marrow, though smaller amounts are found in most tissues, including lung. ^{232}Th decays with a radioactive half-life > 10^{10} years, emitting mostly α-particles. One daughter nuclide is ^{220}Rn, which is constantly exhaled, thereby further exposing the bronchial epitheliums to α-particles (Ishikawa *et al.*, 1992). Thus, studies of Thorotrast-injected individuals may provide information about the nature and the risk of lung carcinoma and other cancers from radon-exposure not related to underground mining. We have assessed the incidence of cancer by linkage with the Danish Cancer Registry among 999 Danish Thorotrast-administered patients and found the risk for lung carcinoma to be significantly elevated when related to that of the general population's standardized morbidity ratio (SMR) of 1.92 (95% confidence interval [95% CI], 1.19–2.93). When relating to a control group of similar non-exposed patients, relative risk (RR) was 1.64 (95% CI, 0.92–2.91). Further, the incidence of malignant tumors of pleura and peritoneum was also significantly increased: SMR, 8.33 (95% CI, 2.69–19.45) and RR, 9.41 (95% CI, 1.05–444.75) (Andersson *et al.*, 1995 *a).*

This elevated risk for lung carcinoma and malignant mesothelioma could be hypothesized to be due to exhaled radon or α-particles from deposited thorium. In the present study, the incidence and histology of lung carcinoma and malignant mesothelioma among individuals in the cohort of Thorotrast-administered patients was studied by histopathologic review.

[9]To whom correspondence and reprint requests should be sent, at Danish Cancer Society, Division for Cancer Epidemiology, Box 839, 49 Strandboulevarden, DK-2100 Copenhagen, Denmark. Fax: 45 35260090.

[10]Present address: National Institute of Occupational Health, Copenhagen, Denmark.

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As specific patterns of *p53* mutations have been reported in lung carcinomas among radon-exposed persons, we also, whenever possible, characterized the spectrum of such mutations in Thorotrast-related lung carcinomas and mesotheliomas. As *p53* mutations have been only infrequently studied in malignant mesothelioma, we further examined these mutations in 11 cases of malignant mesothelioma attributed to asbestos exposure. Finally, as control of the methods used, *p53* mutations were examined in 10 randomly selected cases of SCLC, where the incidence is usually high.

MATERIAL AND METHODS

The Danish Thorotrast study

Details of the patients enrolled in the study were reported earlier (Andersson *et al.*, 1995 *a)*. In summary, 999 (564 males and 435 females) Danish neurological patients injected with Thorotrast during 1935–1947 for cerebral arteriography were identified and followed through 1992 for vital status by means of several administrative registers. Exposure to Thorotrast was verified and the amount injected was documented through reviews of original hospital records for all but 9 patients. The average amount of Thorotrast administered was 18.7 ml (range 8-80 ml). The follow-up period was from the time of injection of Thorotrast to death or 20 January 1992, whichever came first and the average follow-up time was 20.4 years (range 0-54 years).

Histopathological revision of cases with lung carcinoma and malignant mesothelioma

Possible cases of lung carcinoma and malignant mesothelioma among Thorotrast patients were searched for *(i)* by linkage to the Danish Cancer Registry (cases reported as lung carcinoma [primary, not specified as primary and metastatic] and as tumors of pleura or peritoneum); *(ii)* by linkage to the National Hospital Discharge Registry, which keeps information of all diagnoses from hospitals admissions since 1977 (cases with the same diagnoses as for the Cancer Registry which failed Cancer Registry registration); and through a review of *(iii)* death certificates (cases with cancer as cause of death which were not reported to the Cancer Registry); *(iv)* hospital records for patients who died in a hospital and *(v)* autopsy reports (cases not reported to the Cancer Registry as lung carcinoma or malignant mesothelioma but where clinical or pathological information is suggestive for these diseases). For patients with suspected lung carcinoma or malignant mesothelioma, relevant hospital records, pathology reports and available histologic specimens were requested and reviewed by one of us (JV). Cases were classified as proposed by WHO (World Health Organization, 1981). For suspected malignant mesotheliomas, pathology slides were stained with hematoxylin-eosin, van Gieson and periodic acid Schiff ± diastasis. Immunohistochemical stainings were carried out for carcinoembryonic antigen (monoclonal antibody), epithelial antigen (EP4), B72.3 antigen, vimentin and cytokeratin (wide-spectrum screening antibody). Cumulative frequencies of incidence were estimated with Kaplan-Meier methods and differences were evaluated by the log-rank test. Categorical variables were analyzed utilizing the *t* test or χ^2 test.

Analysis of p53 *mutations in lung carcinomas and malignant mesotheliomas in Thorotrast patients and in malignant mesotheliomas in asbestos-exposed patients*

All available paraffin-embedded, formalin-fixed lung tumors and malignant mesotheliomas from Thorotrast-exposed patients were analyzed. Coding and splice site sequences of the *p53* gene in exons 5–8 were amplified by PCR and analyzed by constant denaturant gel electrophoresis and direct sequencing as described previously (Andersson *et al.*, 1995 *b)*. Confirmatory studies of exons 5 and 8 in lung tumors were conducted at another laboratory, using amplification by 2 sequential rounds of PCR as described previously (Lehman *et al.*, 1991) followed by sequencing using a modified version of a method used earlier (Hsu *et al.*, 1991).

Tumor tissues from 11 cases of malignant mesothelioma from asbestos-exposed workers were identified from the files of the Institute of Pathology, Aalborg Sygehus, Denmark, and examined for *p53* mutations, as described earlier (Andersson *et al.*, 1995 *b)*. All 11 patients were industrial workers who had been exposed to asbestos dust for years, mainly working with asbestos-containing roofing or insulation material. None of these patients had had injections with Thorotrast. Autopsies were carried out in 7 cases, all of which revealed asbestos bodies in lung sections as demonstrated by light microscopy.

As control lung tumor samples, paraffin-embedded, formalin-fixed tumor tissues from 10 cases of SCLC were randomly selected from the files of the Institute of Pathology and analyzed for *p53* mutations using the same methods as utilized for tumors from the present study.

No examination of expression of *p53* protein by immunohistochemical methods was performed.

RESULTS

Summary results of the pathological review are given in Table I. The majority of suspected cases were reported to the Cancer Registry. No case without Cancer Registry notification was found on death certificates or in the National Hospital Discharge Registry. Five cases, however, were found by review of hospital records only. The pathology review resulted in only a few reclassifications.

Primary lung carcinoma

Incidence and histology. Diagnoses of 20 cases with primary lung carcinoma were verified (Tables I, II). Eighteen cases had been histologically examined originally, and pathologic specimens from 11 of these were available for restaining. Patients with lung carcinoma did not differ from the whole cohort of Thorotrast-exposed patients with regard to mean age at injection (31 *vs.* 37 years), latency period from injection to diagnosis of lung carcinoma or death from other causes (27 *vs.* 20 years) or amount of Thorotrast injected (19 *vs.* 19 ml) (Table II). The male:female ratio among patients with lung carcinoma was 19:1.

Among the 18 histologically examined tumors, 9 (50%) were SCLC, 4 adenocarcinomas (22%), 4 squamous cell carcinoma (22%) and 1 large cell carcinoma (6%) (Table II). Smoking history was available for 8 patients. Six patients were smokers (3 SCLC, 3 squamous cell carcinomas), and 2 patients had never smoked (1 SCLC, 1 adenocarcinoma). The available material did not locate the carcinomas in the lungs *(i.e.*, peripheral or central).

The cumulative frequency of primary lung carcinoma reached 11.0% (standard error 4.1%; Fig. 1). The temporal pattern for the cumulative frequency of SCLC and non-SCLC did not differ, as seen in Figure 1.

p53 *mutations.* Available tumor tissue allowed analysis of *p53* mutations in 7 cases (4 SCLC, 2 adenocarcinoma, 1 large cell carcinoma). Information on smoking history was available for only 1 patient, a smoker with SCLC. Confirmatory sequencing of exon 5 was performed in *5* tumors (3 SCLC, 2 adenocarcinoma) and of exon 8 in 2 tumors (1 SCLC, 1 adenocarcinoma). A *p53* mutation was discovered in only 1 case, an adenocarcinoma, at codon 266, where GGA^{gly} was substituted with TGA^{stop} on the coding strand. This mutation was observed in tissue from the primary tumor. Due to insufficient amount of tissue, confirmatory studies could be performed only on metastatic lesions which exhibited no mutations.

Analysis of archival tumor tissue from 10 randomly selected patients with SCLC revealed mutations in 4 tumors at codons **138 (GCC → CCC), 156–159 (10 nt deletion CGCGTCCGCG**

TABLE I - REVIEW OF CASES SUSPECTED FOR LUNG CARCINOMA OR MALIGNANT MESOTHELIOMA AMONG DANISH THOROTRAST-ADMINISTERED PATIENTS

Primary source of information	Originally reported diagnostic entity	Basis for original diagnosis	Material secured and reviewed for current study	Study diagnosis based on review of material listed in preceding column
Cancer register	SCLC[1]	Pathology	Pathology report	SCLC
Cancer register	Anaplastic carc.	Pathology	Pathology slides	SCLC
Hospital record	SCLC	Pathology	Pathology slides	SCLC
Cancer register	Oat cell carc.	Pathology	Pathology slides	SCLC
Cancer register	Solid carc.	Pathology	Pathology slides	SCLC
Hospital record	SCLC	Pathology	Pathology slides	SCLC, Also primary liver carc.
Cancer register	Bronchial carc.	Pathology	Pathology slides	SCLC
Cancer register	Anaplastic carc.	Pathology	Pathology slides	SCLC
Cancer register	Anaplastic carc.	Pathology	Pathology report	SCLC
Cancer register	Adenocarc.	Pathology	Pathology report	Adenocarc. of lung
Cancer register	Adenocarc.	Pathology	Pathology report	Adenocarc. of lung
Cancer register	Gigantocellular carc.	Pathology	Pathology slides	Adenocarc. of lung
Cancer register	Adenocarc.	Pathology	Pathology slides	Adenocarc. of lung
Cancer register	Squamous cell carc.	Pathology	Pathology report	Squamous cell carc. of lung
Cancer register	Squamous cell carc.	Pathology	Pathology report	Squamous cell carc. of lung
Cancer register	Squamous cell carc.	Pathology	Pathology reports	Squamous cell carc. of lung
Cancer register	Squamous cell carc.	Pathology	Pathology slides	Squamous cell carc. of lung
Cancer register	Large cell carc.	Pathology	Pathology slides	Large cell carc. of lung
Hospital record	Lung carc., NOS	Clinical	Hospital record	Lung carc., NOS
Cancer register	Lung carc., NOS	Clinical	Death certificate	Lung carc., NOS, Autopsy performed, no histology
Cancer register	Carcinoid of lung	Pathology	Pathology slides	Malignant mesothelioma of pleura and peritoneum
Cancer register	Malignant mesothelioma	Pathology	Pathology slides	Malignant mesothelioma of pleura
Hospital record	Malignant mesothelioma	Pathology	Pathology slides	Malignant mesothelioma of peritoneum
Hospital record	Malignant mesothelioma	Pathology	Pathology slides	Malignant mesothelioma of peritoneum
Cancer register	Malignant mesothelioma	Pathology	Pathology slides	Malignant mesothelioma of peritoneum
Cancer register	Primary liver carc.	Pathology	Pathology slides	Malignant mesothelioma of peritoneum. Also primary liver carc.
Cancer register	Primary liver carc.	Pathology	Pathology slides	Malignant mesothelioma of peritoneum
Cancer register	Lung carc., NOS	Clinical	Pathology slides	Lung lesion most likely metastatic from hepatic angiosarcoma
Cancer register	Sarcoma of lung	Pathology	Pathology report	Lung lesion most likely metastatic from hepatic angiosarcoma
Cancer register	Primary liver carc.	Pathology	Pathology slides	Adenocarc. with unknown site of origin
Cancer register	Lung carc., NOS	Pathology	Pathology slides	Lung lesion most likely metastastic from breast carc.
Cancer register	Adenocarc. of peritoneum	Pathology	Pathology slides	Peritoneal lesion most likely metastatic from hepatic carc.
Cancer register	Adenocarc. of peritoneum	Pathology	Pathology report	Peritoneal lesions most likely metastatic from unknown primary

[1] *Abbreviations:* SCLC, small cell lung cancer; NOS, not otherwise specified; carc., carcinoma; adenocarc., adenocarcinoma.

TABLE II - SELECTED CHARACTERISTICS OF THOROTRAST-ADMINISTERED PATIENTS WITH LUNG CARCINOMA AND MALIGNANT MESOTHELIOMA

Type of cancer	N	Histology reviewed	Age at injection (yr)			Years from injection to diagnosis			Amount of Thorotrast injected, ml		
			Mean	SD[1]	Range	Mean	SD	Range	Mean	SD	Range
Small cell carcinoma	9	7	32	11	16–52	24	12	10–46	19	16	10–40
Adenocarcinoma	4	2	27	15	13–49	30	13	13–42	20	14	10–40
Squamous cell carcinoma	4	1	37	3	31–39	36	12	13–40	22	13	9–40
Large cell carcinoma	1	1	13			49			10		
Unclassified[2]	2	0	38	14	28–48	30		29–31	15	7	10–20
All lung carcinomas	20	11	31	12	13–52	27	12	10–49	19	11	9–40
Malignant mesothelioma	7	7	22	12	6–37	30	5	22–36	27	14	10–50
Whole study population[3]	1,003		37	15	1–73	20	16	0–54	19	11	8–80

[1]SD, standard deviation. -[2]Two cases without histological confirmation .-[3]For the whole study population, the time for diagnosis is substituted with the time of death, emigration or end of follow-up.

leading to a premature stop codon at codon 180), 168 (CAC → GTC) and 249 (AGG→ ATT), respectivelv. Smoking histories for these patients are not known.

Malignant mesothelioma

Incidence and histology. Histopathologic review confirmed 7 cases of malignant mesothelioma. The male:female ratio was 6:1. Only 4 of the cases had been initially histologically confirmed as malignant mesothelioma (Table I). For 6 cases, new slides were prepared and examined immunohistochemitally, while original slides were reviewed in 1 case. Of the 7 cases, 5 were peritoneal (2 epithelial, 1 mesenchymal, 2 biphasic type), 1 was pleural (biphasic type) and 1 was both pleural and peritoneal (biphasic type). The histologic pattern

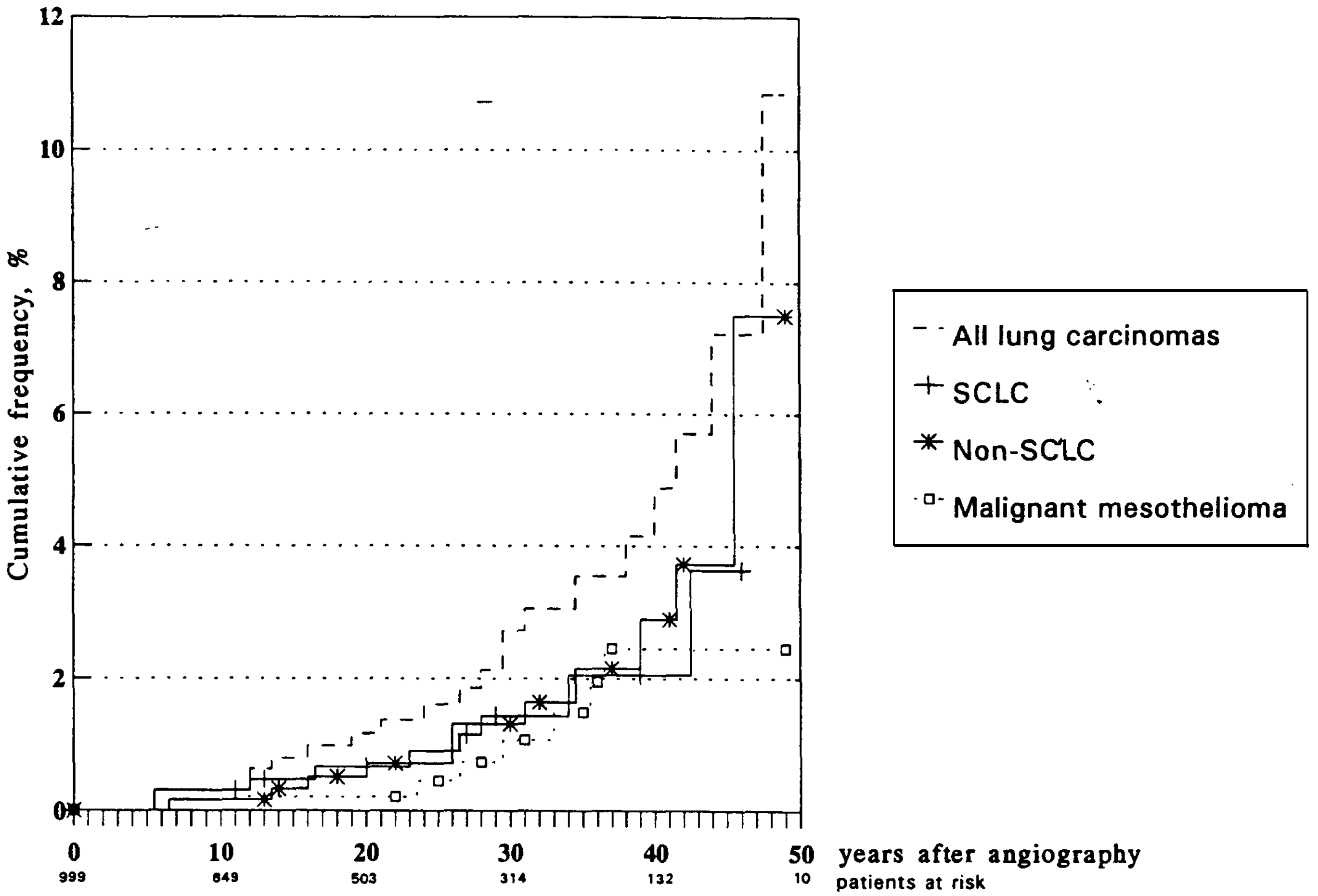

FIGURE 1 - Cumulative frequency (Kaplan-Meier estimate) of small cell lung carcinoma, non-small cell lung carcinoma, all lung carcinomas combined and malignant mesothelioma among Thorotrast-administered patients.

was similar to that of asbestos-related and other mesotheliomas. In 1 case Thorotrast granules were seen in the slide but not adjacent to tumor tissue.

Patients with malignant mesothelioma appeared to have been younger than the whole population of Thorotrast-exposed patients at the time of injection (mean 22 *vs.* 37 years; Table II). The exposure time from injection to diagnosis of cancer or death was relatively long (mean 30 *vs.* 20 years) and the amount of Thorotrast injected was higher (mean 27 *vs.* 19 ml). The cumulative frequency of malignant mesothelioma reached 2.5% (standard error 1.0%; Fig. 1). For patients injected with <=20 ml of Thorotrast (792 patients, 3 cases of malignant mesothelioma), the cumulative frequency was 1.4% (standard error 0.8%) compared to 7.8% (standard error 4.1%) among patients who received >20 ml of Thorotrast (198 patients, 4 cases; log rank test, $p = 0.002$).

p53 *mututions.* Tissues from 5 malignant mesotheliomas, 4 peritoneal (2 epithelial, 1 mesenchymal, 1 biphasic) and one both pleural and peritoneal (biphasic) from Thorotrast-exposed patients were available for *p53* analysis. Further, archival tissue from 11 malignant mesotheliomas, 10 pleural (8 epithelial, 1 mesenchymal, 1 biphasic) and one peritoneal (epithelial) were available for study. No mutation was discovered in any tumor.

DISCUSSION

There are indications that the risk for lung carcinoma is elevated among Thorotrast-administered patients in Denmark (RR, 1.64; Andersson *et al.*, 1995a) and Japan (RR, 1.7; Ishikawa *et al.*, 1992), whereas the risk is not elevated in German patients (RR, 0.75; van Kaick *et al.*, 1995). For malignant mesothelioma, the risk has been demonstrated to be elevated in the Danish (RR, 9.41), the Japanese (1 case among 255 Thorotrast-administered patients, none among 1,630 controls), and the German (9 cases among 2,326 Thorotrast-administered, none among 1,890 controls) studies. Thorotrast is known to be a very potent human carcinogen. Thus, the incidence of cancer at all sites combined, among patients given Thorotrast by intra-arterial injection, is elevated more than 3-fold compared with the general population. This is due mainly to a more than 100-fold risk of liver cancer, but elevated risks are even seen for cancer at several other sites (Andersson *et al.*, 1995a). Both early (reviewed in Wegener, 1979) and recent (Wesch *et al.*, 1983, 1986; Spiethoff *et al.*, 1989; Taylor *et al.*, 1993) experimental studies indicate that the carcinogenic effect, at least as regards the liver, is related to radiation, mostly α-particles from the decay of ^{232}Th, and not to chemical or other factors. Whereas more than 90% of injected $^{232}ThO_2$ is deposited in liver, spleen and bone marrow, measurable amounts (approximately 1.3% of the concentration in liver) are also observed in pulmonary tissue. One daughter nuclide of the decay of ^{232}Th is ^{220}Rn. It has been estimated that approximately 8% of ^{220}Rn emanating from deposits of $^{232}ThO_2$ is exhaled, thus further exposing pulmonary tissue to the potential carcinogenic effects of α-particles (van Kaick *et al.*, 1984).

Histologic types and risk of lung carcinoma

The distribution of lung carcinomas with regard to histological subtype in the present study (Table II) differs significantly from the pattern observed among lung carcinomas in the general population. In a study where all lung carcinomas notified to the Danish Cancer Registry during 1978–1986 were reclassified (Krasnik *et al.*, 1994), the numbers of SCLC, adenocarcinomas, squamous cell carcinomas, large cell carcinomas and others were 248, 263, 455, 114 and 287, respectively, compared to the distribution in the present study: 9, 4, 4, 1 and 2 (χ^2 test, p = 0.03). The difference is due to the relative preponderance of SCLC in the present series, 9/20 = 45% of all tumors compared to 248/1,367 = 18% in the reference material (Fisher's exact test, p = 0.01). This finding is intriguing inasmuch as a similarly significant preponderance of SCLC was observed in the Japanese study (Ishikawa *et al.*, 1992) and among underground miners exposed to radon (National Research Council, 1988). These observations corroborate the notion that radiation-induced lung carcinomas appear more likely to be SCLC, as proposed in a comparative analysis of lung carcinomas among Japanese A-bomb survivors and among uranium miners (Land *et al.*, 1993). Moreover, in the most recent report of cancer incidence among A-bomb survivors, the estimated excess relative risk per Sv for SCLC was 2.07 (95% CI = 0.6–4.6) compared to adenocarcinoma, 1.3 (0.6–2.2); squamous cell carcinoma, 0.81 (0.2–1.8) and others, 0.43 (–0.23–1.71) (Thompson *et al,* 1994).

Risk estimates for lung carcinoma induced by inhalation of radon in dwellings are based on extrapolations from studies of underground miners, who are occupationally exposed to markedly greater concentrations of radon and who experience a high incidence of primary lung carcinoma. The finding that Thorotrast-injected individuals chronically exhale radon might provide a possible mechanism to establish relatively precise dose-response estimates for radon-induced lung carcinoma, as these patients, in contrast to underground miners, are typically not exposed to agents such as silica, arsenic, diesel fumes and others, which may contribute to increased risk (Lubin *et al.*, 1994), though they may be exposed to tobacco. Estimation of radiation dose relevant for pulmonary carcinogenesis from exhaled radon in Thorotrast-administered patients is, however, complicated by several factors. Hoffman *et al.* (1990) and Hornik and Kaul (1995) have provided dose estimates resulting in relative risks for lung carcinoma among Thorotrast-administered patients of 1.6 and 2.2–7, respectively— *i.e.*, elevated risks which have not been observed at least in the German Thorotrast study (van Kaick *et al.*, 1995). This observation has been explained in several ways—e. g., that the models used are inappropriate (Hornik and Kaul, 1995) and that the deposition pattern of inhaled *(i.e.,* underground miners) differs from exhaled *(i.e.,* Thorotrast-exposed) aerosols (Ishikawa *et al.*, 1992). Further, the high incidence of lung carcinoma among underground miners may be influenced by agents other than radon, such as mycotoxins (Venitt and Biggs, 1994) or mine dusts (Ishikawa *et al.*, 1992). This latter theory is supported by the observation that the lung carcinoma risk among underground miners is heavily influenced by arsenic (Lubin *et al.*, 1994), similar to studies in rats in which a significant interactive effect of inhaled silica dust and injected Thorotrast on the incidence and latency period of lung tumors is noted (Spiethoff *et al.*, 1992).

For the 999 patients in the present study who received a mean dose of 18.7 ml Thorotrast and who were alive and at risk for 20.4 years after the administration of Thorotrast, on average, the mean α-particle cumulative tracheo-bronchial dose can be roughly estimated as 0.177 Gy under the assumption that the dose rate is 48.3 x 10^{-5} Gy per ml Thorotrast injected per year (Hoffmann *et al.*, 1990) and allowing for a latency period of 5 years. In the recent compilation of available data on lung carcinoma risk among underground miners exposed to radon, the excess relative risk has, under the assumption of a latency period of 5 years, been estimated to be 0.005 per working level month (WLM, a time-integrated exposure measure defined as the product of time, taken to be 170 hr. and working level [WL], where 1 WL equals any combination of radon progeny in 1 l of air, resulting in the ultimate emission of 130,000 MeV of energy from α-particles; Lubin *et al.*, 1994). If applying this estimate to data from the present study and assuming that 1 WLM is equal to 5 x 10^{-3} Gy lung dose (Hofmann *et al.*, 1990), the excess relative risk can be very roughly estimated as 0.177 and thus the relative risk as 1.177. This value is compatible with the relative risk for lung carcinoma based on cancer registry data of 1.64 (95% CI = 0.92–2.91) found among the patients in the present study (Andersson *et al.*, 1995a).

Incidence of malignant mesothelioma

The most frequent known cause of malignant mesothelioma, which is normally a very rare disease, is asbestos exposure. However, a high frequency of malignant mesothelioma of peritoneum has been observed following i.p. injection of α-particle-emitting $^{239}PuO_2$ in rats (Sanders and Jackson, 1972). In addition, case reports have described malignant pleural as well as peritoneal mesotheliomas occurring both after radiation treatment (X-ray or photons) for different malignant and benign conditions (Wilm's tumor, cervical cancer, seminoma, Hodgkin's disease, teratoma, breast cancer, and goiter; for references see Talcott and Antman, 1988; Austin *et al.*, 1986; Beir *et al.*, 1984) and following topical application (in contrast to intravascular injection) of Thorotrast (Dahlgren 1967; Maurer and Egloff 1975). Malignant mesotheliomas have typically not been observed in the major epidemiologic radiation carcinogenesis studies, but interestingly, in the German Thorotrast study, 4 peritoneal and 5 pleural mesotheliomas were diagnosed among 2,326 Thorotrast-exposed persons compared to no cases among 1,890 controls (van Kaick *et al.*, 1995). In the Japanese Thorotrast study, 1 case of peritoneal mesothelioma was described among 255 Thorotrast-exposed patients compared to none among 1,630 controls (Mori and Kato, 1991). In the present study, 7 cases of malignant mesothelioma occurred. Based on incidence rates of the Danish Cancer Registry, only 0.6 cases of malignant tumors of pleura and peritoneum (including both mesotheliomas and tumors with other histologies) would have been expected (Andersson *et al.*, 1995a). The observed numbers of malignant mesotheliomas in this study are the result of a patho-anatomical review, while the incidence rates are derived from notifications to the Cancer Registry. Nevertheless, it is clear that the risk of malignant mesothelioma among Thorotrast-exposed patients is greatly increased, and it can be hypothesized that these tumors were induced by α-particles from Thorotrast deposited in organs (liver, spleen, abdominal and thoracic lymph nodes) adjacent to pleura or peritoneum. This hypothesis is further supported by the positive association between radiation dose and occurrence of malignant mesothelioma inasmuch as the time from injection of Thorotrast to death or diagnosis was longer among patients with malignant mesothelioma than the whole population and the injected amount of Thorotrast was significantly higher among patients with malignant mesothelioma than the whole cohort. Further, the prevalence of peritoneal mesotheliomas in our study, in contrast to the general population, speaks in favor of a relation to radiation as mesothelium of peritoneum is more likely to be exposed to α-particles, *e.g.*, from Thorotrast deposited subcapsulary in liver, spleen and lymph nodes than the mesothelium of the pleura. The thickness of the liver capsule, which measures approximately 50 μm, and the tissue range, up to approximately 50 μm, of α-particles derived from the decay of ^{232}Th are also compatible with this hypothesis. The data of our study cannot, however, rule out that the mesotheliomas have arisen as a consequence of non-radiation effects of Thorotrast. Malignant mesothelioma can be induced experimentally by application of a number of synthetic and natural minerals (Talcott and Antman, 1988).

p53 *mutations in lung carcinomas and malignant mesotheliomas*

The *p53* suppressor gene is considered to play a central role in the control of cell replication. *p53* mutations have been demonstrated in high frequencies in several types of tumor, and the spectrum of mutations may provide clues to the etiology and pathogenesis of the disorders (Greenblatt *et al.*, 1994).

Examination of available material for mutations of exons 5–8 of the *p53* tumor suppressor gene revealed no mutation in 4 cases with SCLC) and 1 mutation among 3 non-SCLC. This is lower than expected based on the published prevalence of *p53* mutations in lung carcinoma, which for all lung carcinomas is 56% and for SCLC 70% (Greenblatt *et al.*, 1994). Our findings are also in contrast to observations of specific mutational spectra in lung carcinomas from radon-exposed underground miners (Greenblatt *et al.*, 1994) or following radiation treatment (De Benedetti *et al.*, 1994) but in accord with results of *p53* analyses of α -particle-induced liver tumors among Danish Thorotrast-exposed persons, where the frequency of mutations was also lower than expected (Andersson *et al.*, 1995b). The finding of a *p53* mutation in a primary tumor but not a metastatic lesion may be explained by considering them to be synchronous, independent tumors with different genetic lesions. Such differences have been demonstrated in a series of 11 synchronous lesions in 5 lung carcinoma patients with multiple primary tumors (Sozzi *et al.*, 1995).

Examination of 5 malignant mesotheliomas among Thorotrast-exposed patients with increased risk for this disease presumed to be due to α -particles and 11 such cases presumably induced by exposure to asbestos fibers did not reveal any point mutations of exons 5–8 of the *p53* gene. Normally, malignant mesotheliomas are characterized by a high frequency of complex chromosomal aberrations (Popescu *et al.*, 1988; Tiainen *et al.*, 1988; Pelin-Enlund *et al.*, 1990). To our knowledge, no paraffin-embedded, formalin-fixed malignant mesothelioma tissue has previously been examined for *p53* mutations. In 23 cell lines established from malignant mesotheliomas, *p53* mutations have been discovered in 5 cases (22%) (Cote *et al.*, 1991; Metcalf *et al.*, 1992).

Thus, the results of *p53* analyses in the present study of lung carcinomas and malignant mesotheliomas possibly induced by α -particles do not support previous suggestions that specific point mutations or mutational patterns are important in the pathogenesis of these disorders. According to experimental evidence, the genomic effects of exposure to α -particles would often be gross chromosomal aberrations and large deletions rather than point mutations. The methods used for *p53* analysis in the present study would not detect large deletions, so even though the frequency of point mutations was low it can not be precluded that inactivation of *p53* by large deletions is important in the pathogenesis of α -particle-induced malignancies. A subject for future research on supposedly radiation-induced malignancies could be the characterization of major chromosomal aberrations.

In conclusion, our findings support a relationship between radiation from α -particles and both SCLC histology and the development of mesothelioma; further, a dose-response relationship between Thorotrast exposure and malignant mesothelioma incidence may exist.

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